Decomposition of *S*-nitrosothiols: the effects of added thiols

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The Cu$^+$ (added as Cu^{2+}) mediated decomposition of the five *S*-nitrosothiols, derived from penicillamine, cysteamine, thiomalic acid, *N*-acetylpenicillamine and cysteine have been examined kinetically in the presence of varying amounts of the corresponding thiols. Large differences in behaviour were found. In some cases, reactions were catalysed by added thiols, whereas in others, stability was conferred, often resulting in the appearance of quite large induction effects. The results are explained in terms of the dual function of the thiol (as thiolate), (*a*) as a reducing agent generating Cu$^+$, and (*b*) as a complexing agent for Cu^{2+}, when it is then less available for reduction. The balance of these two effects depends on the structure and concentration of the added thiol. The findings were supported by examining the two effects separately, using ascorbate as a reducing agent, and ethylenediaminetetraacetic acid as the complexing agent. For penicillamine, cysteine and thiomalic acid, the Cu^{2+} complexes were identified from their UV spectra, and their decomposition was followed kinetically.

It is now clear[1] that the decomposition of *S*-nitrosothiols (or thionitrites) in aqueous solution [eqn. (1)], is brought about by

$$2RSNO = RSSR + 2NO \qquad (1)$$
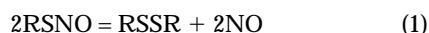
Cu$^+$, generated by reduction of Cu^{2+} by thiolate ion [eqn. (2)].[2]

$$2Cu^{2+} + 2RS^- = 2Cu^+ + RSSR \qquad (2)$$
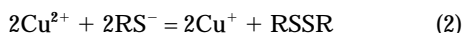
Often there is enough Cu^{2+} present in the distilled water–buffer components to effect reaction, whereas in other cases it can be added in low concentration (typically 1×10^{-6} to 1×10^{-5} mol dm^{-3}) as a Cu^{II} salt. It has also been shown[3] that copper(II) bound to peptides or proteins can be reduced in the same way, allowing the possibility that NO release from RSNO compounds can occur *in vivo*. When metal ions are removed, for example by ethylenediaminetetraacetic acid (EDTA), or when Cu$^+$ is removed specifically with neocuproine,[1] then no reaction occurs, except for a photochemical decomposition (if in the presence of light), and a very slow thermal decomposition.

Until recently, it was not clear what the source of the thiolate anion was, and suggestions were made involving (*a*) the presence of some thiol as an impurity in the nitrosothiol sample, and (*b*) a small extent of hydrolysis of the nitrosothiol. Now,[4] it is known that the nitrosation of thiols [eqn. (3)] is sufficiently

$$RSH + HNO_2 = RSNO + H_2O \qquad (3)$$

reversible to ensure that there is usually enough thiol present to yield enough thiolate in the buffer (pH 7.4) to bring about the reduction of Cu^{2+}. For example in the case of penicillamine the equilibrium constant for RSNO formation [eqn. (3)] is *ca.* 5×10^5 dm^3 mol^{-1}, which means that when equimolar (6.7×10^{-4} mol dm^{-3}) amounts of the thiol and nitrous acid are mixed, thiol remains at a concentration of *ca.* 3.5×10^{-5} mol dm^{-3} at equilibrium. Usually, the [Cu^{2+}] is less than this, and in the absence of any complexation effect, reduction would occur readily under these conditions.

There are a number of references in the biological literature where it is claimed that the addition of thiolate enhances nitrosothiol decomposition, although there are also examples where the reverse is the case, *i.e.* that thiolate addition stabilises

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nitrosothiols in solution. In part, we have offered an explanation,[1] for the reactions of one nitrosothiol, *S*-nitroso-*N*-acetylpenicillamine (SNAP), that at low concentration of added *N*-acetylpenicillamine (NAP), the thiolate is acting as a reducing agent for Cu^{2+}, (catalysis by added thiol), whereas at higher concentration of added NAP, complexation of Cu^{2+} occurs rendering it less available for reduction (stabilisation by added thiol).

In order to test these ideas more quantitatively, and also to establish their wider generality, we have examined the decomposition of the five *S*-nitrosothiols derived from penicillamine (**1**), cysteamine (**2**), thiomalic acid (mercaptosuccinic acid) (**3**), NAP (**4**) and cysteine (**5**), in the presence of varying amounts of the corresponding thiols.
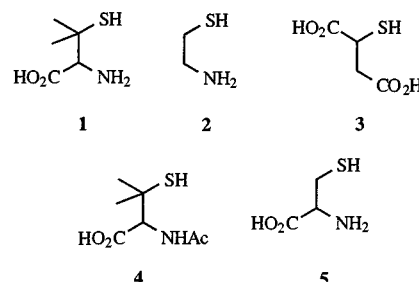

Results and discussion

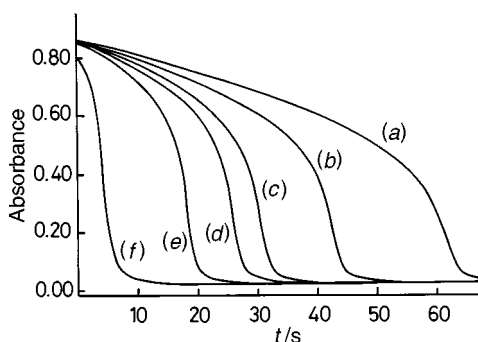### (*a*) *S*-Nitrosopenicillamine and *S*-nitrosocysteamine

In the first set of experiments we measured the disappearance of the absorbance due to the nitrosothiol, **1**, which was generated in solution by nitrosation of penicillamine (PEN), for five solutions, ranging from solutions containing an excess of PEN to those containing a slight excess of nitrous acid. The results, shown as absorbance–time plots for the disappearance of RSNO (Fig. 1) show a large difference in reactivity pattern. When PEN is in excess there is a large time period when reaction is very slow (almost an induction period), and later, reaction quite suddenly becomes much faster. As the PEN excess is decreased the induction period decreases, until a limit is reached, where nitrous acid is in slight excess, when reaction is quite rapid and is close to a first-order pattern with no induction period.

A similar series of curves was obtained (Fig. 2) when **1**, generated in acid solution (where it is reasonably stable), was added
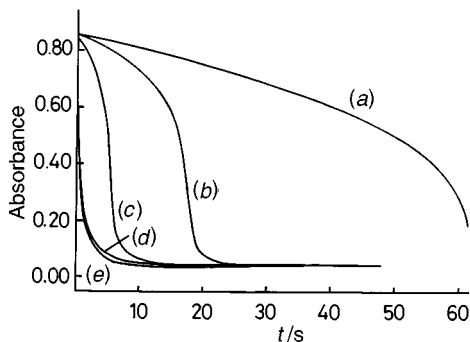
Fig. 1 Absorbance–time plots for the decomposition of *S*-nitrosopenicillamine $(1 \times 10^{-3} \text{ mol dm}^{-3})$ containing added Cu^{2+} $(1 \times 10^{-5} \text{ mol dm}^{-3})$, with (*a*) excess PEN $(1.6 \times 10^{-4} \text{ mol dm}^{-3})$, (*b*) excess PEN $(8 \times 10^{-5} \text{ mol dm}^{-3})$, (*c*) formally no excess PEN, (*d*) excess NO_2^- $(9 \times 10^{-5} \text{ mol dm}^{-3})$ and (*e*) excess NO_2^- $(1.8 \times 10^{-4} \text{ mol dm}^{-3})$
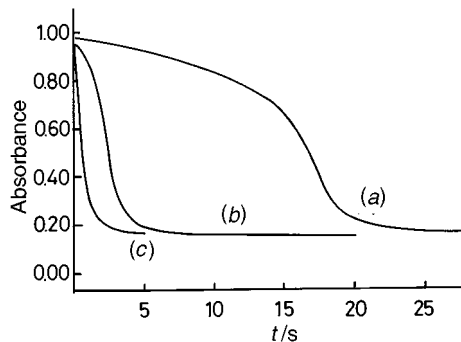

Fig. 2 Absorbance–time plots for the decomposition of *S*-nitrosopenicillamine $(1 \times 10^{-3} \text{ mol dm}^{-3})$ containing added Cu^{2+} $(1 \times 10^{-5} \text{ mol dm}^{-3})$ and excess PEN $(1.6 \times 10^{-4} \text{ mol dm}^{-3})$, when the RSNO–RSH solution had been left standing before addition of the copper and buffer for, (*a*)–(*f*) 25, 40, 50, 60, 90 and 150 min

to the copper–buffer solution, after the nitrosothiol solution had been allowed to stand for different times, up to 150 min before addition to the copper–buffer solution. Under these conditions the loss of nitrosothiol is quite small. Reaction becomes faster, and the induction period decreases, as the standing time of the solution of the nitrosothiol increases.

The results shown in Figs. 1 and 2 can readily be explained if we assume that the presence of excess PEN is complexing Cu^{2+}, making it less available for reduction to Cu^+. Eventually, some Cu^+ will be formed, and since the copper is regenerated in the overall reaction (*i.e.* its action is catalytic),[5] reaction will then proceed rapidly. In the case of the data in Fig. 2, the results make sense only if it is assumed that on standing, the thiol is gradually oxidised to the disulfide. Then, in effect, Figs. 1 and 2 both represent series of experiments with a decreasing thiol concentration for, in each case, the curves moving from right to left. We were able to check this readily by measuring the thiol concentration (using Ellman's reagent), as the RSNO solution was allowed to stand initially in the presence of a large excess of PEN: the results (shown in Table 1) showed a smooth decrease in [PEN] with time which followed the first-order rate law quite well. Because of this marked dependence of the reaction profile upon the 'age' of the nitrosothiol in acid solution, all other measurements in any one series of experiments were made after a fixed time (usually 10 min) after the generation of the nitrosothiol solution.

The effect of changing the $[Cu^{2+}]$ was also quite dramatic, as shown in Fig. 3. As the copper concentration is increased, the reaction becomes much faster, and the induction period progressively disappears. Thus the effect of increasing the copper concentration is to favour the formation of Cu^+, rather than to favour its complexation with thiolate, possibly because of a

Fig. 3 Effect of the $[Cu^{2+}]$ on the absorbance–time plots for the decomposition of *S*-nitrosopenicillamine $(1 \times 10^{-3} \text{ mol dm}^{-3})$, (*a*) $[Cu^{2+}] 5 \times 10^{-6}$, (*b*) $[Cu^{2+}] 1 \times 10^{-5}$ and (*c*) $[Cu^{2+}] 2 \times 10^{-5} \text{ mol dm}^{-3}$

Table 1 Decrease in the thiol concentration[a] from a solution of *S*-nitrosopenicillamine $(2.0 \times 10^{-4} \text{ mol dm}^{-3})$ containing initially penicillamine $(3.9 \times 10^{-5} \text{ mol dm}^{-3})$, PEN on standing in the air

t/min	Absorbance (412 nm)	$[PEN]/10^{-5} \text{ mol dm}^{-3}$
15	0.455	3.21
30	0.425	2.99
45	0.398	2.79
60	0.385	2.69
75	0.368	2.57
90	0.355	2.47
120	0.323	2.24
180	0.285	1.95

[a] Using the Ellman reagent.[10]

saturation effect. The reduction of Cu^{2+} by thiolate ion has been examined, using EPR techniques and UV measurements, by a number of workers, notably Cavallini and co-workers,[6] and more recently Gilbert and co-workers.[7] Evidence was presented in favour of a 2 : 1 thiol : Cu^{2+} complex, as in structure **6**, for the


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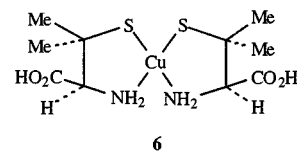
complex with PEN. Similar structures were proposed for corresponding complexes with cysteine and mercaptoethanoic acid. We suggest that our results are best interpreted in terms of eqns. (4) and (5) where the dithiolate complex is formed revers-
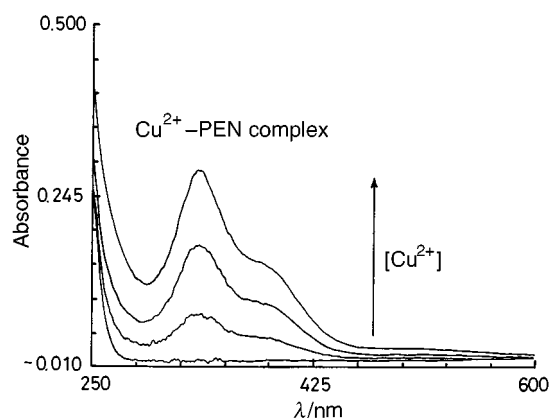
$$Cu^{2+} + 2RS^- \rightleftharpoons \text{Dithiolate complex} \quad (4)$$

$$Cu^{2+} + RS^- \longrightarrow Cu^+ + 0.5RSSR \quad (5)$$
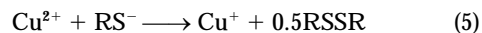
ibly, and that Cu^+ is formed in a parallel reaction of Cu^{2+} with one thiolate ion. In a sense the complexing function of PEN is akin to that of EDTA, and experiments with added EDTA, when there is no excess PEN present show very similar absorbance–time plots (Fig. 4) to those shown in Fig. 1.

We find UV spectra from mixtures of PEN and Cu^{2+}, (shown in Figs. 5 and 6) which are very similar to that found by Cavallini *et al.* for the corresponding cysteine complex,[6] and by Gilbert *et al.* for other thiols. Fig. 5 shows the increasing absorbance (with a maximum at 333 nm) due to the complex as the $[Cu^{2+}]$ is increased from 0 to $5 \times 10^{-5} \text{ mol dm}^{-3}$, when added to PEN $(1 \times 10^{-3} \text{ mol dm}^{-3})$. Fig. 6 shows the decomposition of the complex (as the copper is reduced to Cu^+) at 3 min intervals. Whilst over the years, a number of structures have been proposed for Cu^{2+}–thiol complexes, the most recent analysis by EPR suggests that the complexes which have a UV absorbance
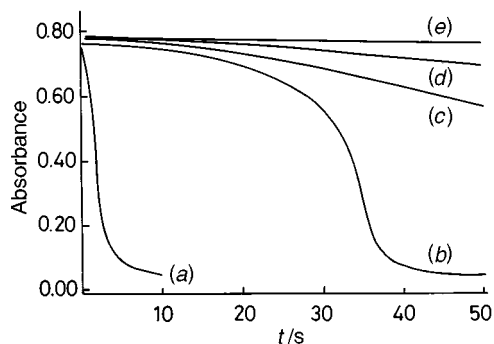
Fig. 4 Effect of added EDTA on the absorbance–time plots for the decomposition of *S*-nitrosopenicillamine prepared *in situ* with a slight excess of HNO$_2$ and containing added Cu^{2+} (1 × 10^{-5} mol dm^{-3}); increasing [EDTA] (*a*)–(*d*), 0, 1 × 10^{-5}, 2 × 10^{-5} and 5 × 10^{-5} mol dm^{-3}, (*e*) no added Cu^{2+} and no added EDTA

Fig. 5 UV spectra showing the Cu^{2+}–PEN complex with increasing added [Cu^{2+}] 0–5 × 10^{-5} mol dm^{-3}

Fig. 6 UV spectra showing the decay of the Cu^{2+}–PEN complex with time

Fig. 7 Formation of the Cu$^+$–neocuproine complex from PEN (1 × 10^{-3} mol dm^{-3}), Cu^{2+} (5 × 10^{-5} mol dm^{-3}) and neocuproine (1 × 10^{-4} mol dm^{-3})

Fig. 8 Absorbance–time plots for the decomposition of *S*-nitrosopenicillamine, prepared *in situ* with a slight excess of HNO$_2$, as a function of the standing time (15–90 min) of the solution before Cu^{2+} and buffer addition
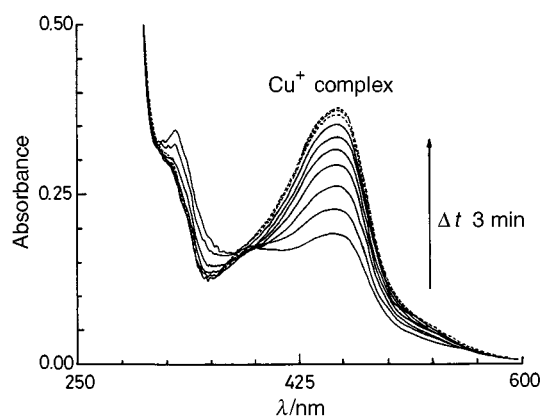
ca. 333 nm, have two *cis*-chelating thiol groups bound to copper(II) as shown in structure **6** for the complex formed from PEN.[7] At higher thiolate concentrations the yellow complex (absorbance maximum 333 nm) is converted to a violet complex which is believed[8] to have a structure involving mixed valence (Cu^{II}–Cu^{I}) forms of copper.

If the complex is formed reversibly from two thiolate ions [eqn. (4)] and reduction of Cu^{2+} involves one thiolate ion [eqn. (5)], then the reaction profile will depend on [RS$^-$], in that at high [RS$^-$], complexation will be the more dominant, and the rate of Cu$^+$ formation will be much reduced, whereas at low [RS$^-$], Cu$^+$ will be more favoured since complexation of Cu^{2+} will be less extensive. This then explains at least qualitatively the experimental absorbance–time profiles, which cannot be explained in terms of the complex being an intermediate in the reduction of Cu^{2+} to Cu$^+$. We have demonstrated that the Cu^{2+}–PEN complex leads to Cu$^+$ formation by the addition of the Cu$^+$-specific chelator neocuproine[9] to a solution of the complex at pH 7.4. We observe (Fig. 7) the fairly rapid build-up of the absorbance at 453 nm, characteristic of the Cu$^+$ complex with neocuproine. Rather different profiles were obtained when solutions of RSNO generated (in acid solution) with excess nitrous acid were allowed to stand for a range of times before addition of the buffer–Cu^{2+} solution. They are shown in Fig. 8. There is no induction period, and reaction becomes slower as the standing time of the RSNO solution increases. Now the [RS$^-$] is very low and so thiolate primarily acts as a reducing agent (with very little complexation of the copper), and on standing the thiol is slowly oxidised to the disulfide, thus reducing the rate of Cu$^+$ formation.
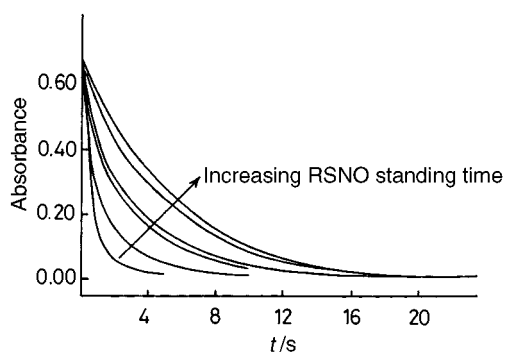
The *S*-nitroso derivative of cysteamine (**2**) behaved in a very similar fashion to that derived from **1**. We obtained absorbance–time plots for the decomposition which were very similar to those in Figs. 1 and 2. There was however, no clear band at *ca.* 333 nm, when Cu^{2+} was added to cysteamine, but there was some evidence of complex formation *via* shoulders at 250 and 290 nm. Addition of neocuproine blocked the RSNO decomposition, and we observed formation of the Cu$^+$–neocuproine complex as before at 453 nm. All of the evidence suggests that although cysteamine behaves in a similar way to PEN, it is not quite such a good chelator of Cu^{2+}.

(*b*) *S*-Nitrosothiomalic acid

This nitrosothiol was generated *in situ* from the thiol **3** and its decomposition studied as in section (*a*). The absorbance–time plots for solutions made with an excess of thiol through to those with excess nitrous acid are shown in Fig. 9. There are
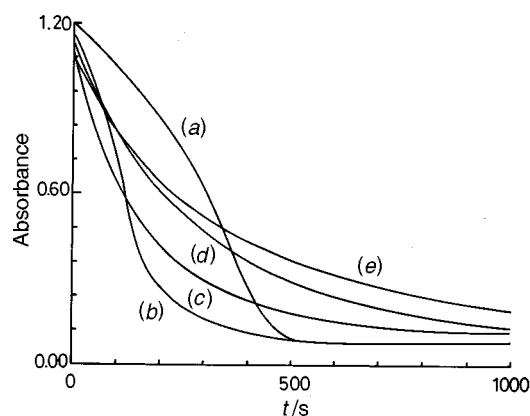
Fig. 9 Absorbance–time plots for the decomposition of S-nitrosothiomalic acid containing added Cu^{2+} (1×10^{-5} mol dm^{-3}), prepared with (*a*) excess thiomalic acid (2.1×10^{-4} mol dm^{-3}), (*b*) excess thiomalic acid (1×10^{-4} mol dm^{-3}), (*c*) equimolar thiomalic acid and HNO$_2$, (*d*) excess HNO$_2$ (1×10^{-4} mol dm^{-3}) and (*e*) excess HNO$_2$ (5×10^{-4} mol dm^{-3})
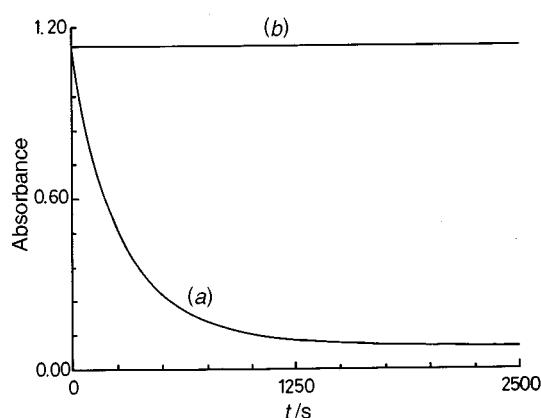

Fig. 10 Decomposition of S-nitrosothiomalic acid, prepared with an excess of HNO$_2$, in the presence of added Cu^{2+}, (*a*) without added EDTA, (*b*) with added EDTA (2×10^{-5} mol dm^{-3})

quite striking similarities here between the pattern shown in (*a*) by the nitrosothiol derived from PEN, but also significant differences. With the highest [excess thiol], initial reaction is the slowest in the group, but there is nothing like as long a quasi-induction period as in (*a*). Thereafter as the excess thiol concentration decreases the rate increases a little and later decreases. The pattern is readily explicable in terms of the dual action of the thiol in (*a*). At the highest excess of thiol, complexation occurs (probably again to give the Cu^{2+}–dithiolate complex), decreasing the concentration of thiolate available for Cu^{2+} reduction. This effect is reduced as the excess thiol concentration is lowered, increasing the overall reaction rate (of RSNO decomposition), until at very low [thiol] the rate becomes slower again as the rate of Cu^{2+} reduction is lowered. These results suggest that the equilibrium constant for copper complexation is much smaller for thiomalic acid than it is for PEN.

We checked that copper is in fact involved for this substrate, by comparison of the reaction profiles in the presence and absence of the metal ion chelator EDTA. The result is dramatic (Fig. 10). For reaction carried out on RSNO samples prepared with a 2:1 excess of HNO$_2$ over RSH, (when the concentration of free thiol will be very low), the presence of EDTA completely blocks reaction.

As in (*a*), we were able to generate UV spectra which we attribute to the $Cu^{2+}(RS^-)_2$ complex. They are shown in Fig. 11 for solutions containing initially Cu^{2+} (5×10^{-5} mol dm^{-3}), and increasing concentrations of thiomalic acid (1×10^{-3} to 5.7×10^{-3} mol dm^{-3}). Approximate calculations suggest that the equilibrium constant for complex formation [eqn. (4)] is *ca.*
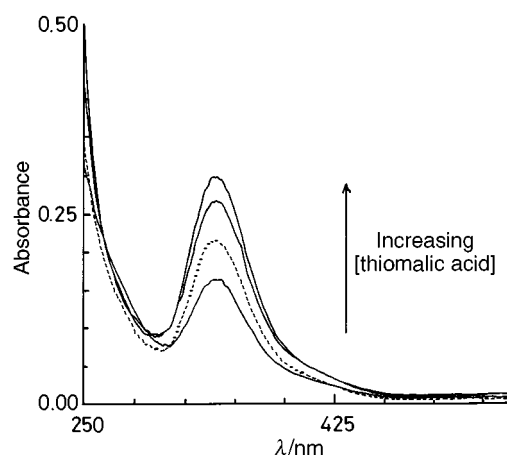
Fig. 11 UV spectra of the Cu^{2+} complex with thiomalic acid, as a function of [thiomalic acid] (1–6×10^{-3} mol dm^{-3}) at constant [Cu^{2+}] (5×10^{-5} mol dm^{-3})

5×10^5 dm^6 mol^{-2}. The decomposition pattern of this complex, leading to Cu^+ and RSSR formation, was similar to that found in (*a*) which is shown in Fig. 6. We have not in this paper pursued the mechanism of this step. The structure of the Cu^{2+}–thiol complex is likely to be similar to that shown in structure **6**, *i.e.* the dithiolate complex. Further quantitative work in this area is underway.
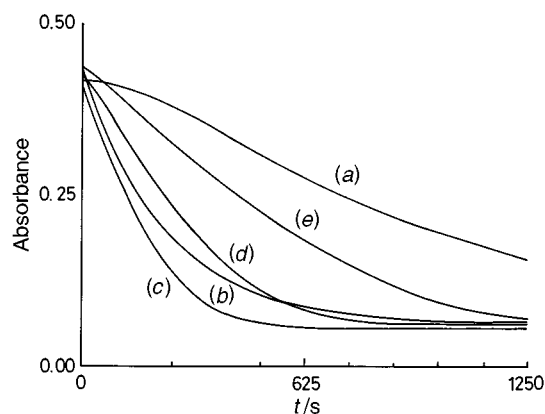
(*c*) *S*-Nitrosocysteine
The behaviour of this nitrosothiol was similar in some ways to that found for S-nitrosothiomalic acid discussed in section (*b*). When the nitrosothiol was generated in the presence of 50% excess of nitrous acid [Fig. 12(*a*)], there was clear evidence of an induction period, which in this work seems to be associated with Cu^{2+}–thiol complex formation. With less (or formally no) excess thiol present the traces all suggest, initially at least, that the reaction is zero-order with respect to [RSNO]. This has been noted previously [5] for some nitrosothiols, under certain conditions, and has been interpreted in terms of the rate-limiting reduction $Cu^{2+} \longrightarrow Cu^+$, which occurs when the reaction of Cu^+ with RSNO is particularly fast because of favourable structural factors. As for the other nitrosothiols, decomposition was virtually halted by the presence of EDTA at a concentration a little in excess over the [Cu^{2+}], and the Cu^+–neocuproine complex was readily generated from cysteine (1×10^{-3} mol dm^{-3}), neocuproine (1×10^{-4} mol dm^{-3}) and Cu^{2+} (5×10^{-5} mol dm^{-3}). Spectra of the Cu^{2+}–dithiolate were obtained which were very similar to those shown in Figs. 4 and 5 for PEN and also to the literature spectra,[6] with an absorbance maximum at 333 nm and a shoulder at *ca.* 400 nm. In contrast to the others, however, the complex only formed at pH values >8, and its decomposition rate was very much greater.

Addition of ascorbic acid (in the concentration range 2.6–10×10^{-7} mol dm^{-3}) as an alternative reducing agent to the thiolate ion produced the expected increase in the rate of RSNO decomposition; much the same effect was produced by the addition of very low concentrations of cysteine (1–5×10^{-7} mol dm^{-3}).

(*d*) *S*-Nitroso-*N*-acetyl-penicillamine (SNAP)
The effect of the presence of *N*-acetyl-penicillamine (NAP) **4** on the rate of decomposition of SNAP is dramatic and is shown as absorbance–time plots in Fig. 13. When there is very little thiol present (when SNAP was prepared *in situ* using a 2.5-fold excess of nitrous acid over NAP), *i.e.* Fig. 13(*a*), no significant decomposition of SNAP occurred (even in the presence of added [Cu^{2+}], 1×10^{-5} mol dm^{-3}) over a 20 min period. When the excess nitrous acid was reduced to a 1.1-fold excess [Fig. 13(*b*)], reaction occurred readily with a half-life of *ca.* 4.5 min,
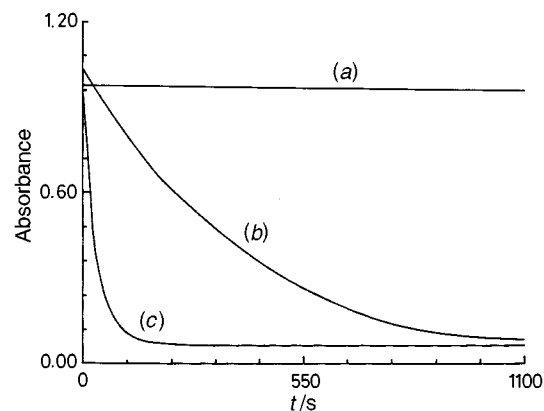
Fig. 12 Absorbance–time plots for the decomposition of *S*-nitrosocysteine (4.7×10^{-4} mol dm^{-3}) prepared *in situ* with (*a*) an excess of HNO$_2$ (2.3×10^{-4} mol dm^{-3}), (*b*) an excess of HNO$_2$ (9.3×10^{-5} mol dm^{-3}), (*c*) equimolar thiol and HNO$_2$, (*d*) an excess of cysteine (2.3×10^{-5} mol dm^{-3}) and (*e*) an excess of cysteine (9.3×10^{-5} mol dm^{-3})

Fig. 13 Absorbance–time plots for the decomposition of SNAP (1×10^{-3} mol dm^{-3}) in the presence of added Cu^{2+} (1×10^{-5} mol dm^{-3}), prepared *in situ* with (*a*) excess HNO$_2$ (2×10^{-3} mol dm^{-3}), (*b*) equimolar thiol and HNO$_2$ and (*c*) excess thiol (1×10^{-3} mol dm^{-3})

Fig. 14 Absorbance–time plots for the decomposition of SNAP (4.66×10^{-3} mol dm^{-3}) with added Cu^{2+} (1×10^{-5} mol dm^{-3}) as a function of added ascorbic acid (0–2×10^{-5} mol dm^{-3})

which was reduced further to *ca.* 24 s when SNAP was prepared with a 1.2-fold excess of NAP over the nitrous acid [Fig. 13(*c*)]. Clearly, the [NAP] has a profound effect on the decomposition rate of SNAP. The same effect is observed (as expected) if SNAP is prepared with equimolar amounts of NAP and nitrous acid, and then increasing quantities of NAP are added to the reaction mixtures. The ageing effect of the SNAP solution, prior to the addition of the buffer and Cu^{2+} is also significant: the longer the solution is allowed to stand the slower is the decomposition. This observation is also consistent with the proposal that the reaction rate is much reduced as the [NAP] is reduced, since NAP is effectively removed from solution by oxidation, on standing. All of these results show that for the decomposition of SNAP, NAP is acting solely as a reducing agent generating Cu$^+$, and plays no (or very little) part as a complexing agent for Cu^{2+}, in marked contrast to the behaviour of PEN. It is clear therefore that *N*-acetylation of PEN reduces its complexing ability for Cu^{2+} enormously. In confirmation of this we were unable to obtain any spectral evidence (in the 333 nm region) for complexation, as we had done previously for PEN, cysteine and thiomalic acid. Similarly, there was no spectral evidence of Cu^{2+} complexation with *N*-acetyl cysteine (not shown).

If, as seems likely, NAP is acting solely as a reducing agent (in the concentration range used), in the generation of Cu$^+$, then the same effect should be observed in principle, from other reducing agents. In this work we have looked only at ascorbic acid in this context. The addition of ascorbic acid in the concentration range 1×10^{-6} to 2×10^{-5} mol dm^{-3} did indeed progressively increase the rate of SNAP decomposition. The absorbance–time data are shown in Fig. 14. Very similar plots (not shown) were obtained by the addition of NAP in the same concentration range, suggesting that NAP and ascorbic acid possess approximately equal reducing properties.

We established that NAP (4.3×10^{-4} mol dm^3) generates Cu$^+$ quantitatively from Cu^{2+} (5×10^{-5} mol dm^{-3}) in buffer pH 7.4, by measurement of the spectrum of the Cu$^+$ complex formed when neocuproine (1.3×10^{-4} mol dm^{-3}) is added. This has now been shown for a range of thiol structures, and can be regarded as a general reaction.

Since this work was completed, we became aware of a paper by Komiyama and Fujimori,[11] that showed that a direct reaction occurs between *S*-nitrosocysteine and quite high concentrations of cysteine, under conditions where metal ions were carefully excluded. The reactions are much slower than we find for the copper catalysed reactions, there are no curious induction effects, and a second-order rate constant was reported. The reaction products were, however, not analysed. We have
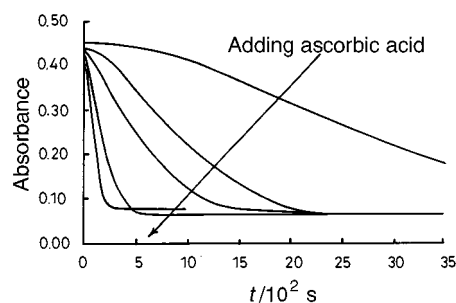
repeated these experiments at pH 7.4 and at 25 °C in the presence of EDTA (1×10^{-4} mol dm^{-3}), working with a range of cysteine concentrations of 0.01–0.10 mol dm^{-3} with a *S*-nitrosocysteine concentration of 1×10^{-3} mol dm^{-3} and obtained a value of 1.1×10^{-2} dm^3 mol^{-1} s^{-1}, which agrees reasonably with the literature value of 3.10×10^{-2} dm^3 mol^{-1} s^{-1} at 37 °C. We have briefly analysed the products for nitrite ion (the product of nitric oxide oxidation and hydrolysis) using the Griess reagent, and find that [NO$_2^-$] drops from 72% at zero added cysteine (the slow thermal decomposition of RSNO), rapidly to 8% at 0.1 mol dm^{-3}. It seems very likely that nitric oxide is therefore not the product of this reaction when Cu^{2+} is eliminated from the solution. Possible products are N$_2$O, derived from the elimination of HNO, or possibly NH$_3$ as has been reported recently by Singh *et al.*[12] from the corresponding reaction of *S*-nitrosoglutathione with high concentrations of glutathione. More work is in progress in this area.

Experimental

All materials used were of the highest purity grade and were used as such. Absorbance–time plots were obtained at *ca.* 340 nm, following the disappearance of the absorbance due to the nitrosothiol, either in a conventional spectrophotometer, or for the faster reactions, in a stopped-flow spectrophotometer. Analyses of Cu$^+$ generated from Cu^{2+} and thiols using the neocuproine reagent were carried out as previously described. Thiol analyses were performed using Ellman's reagent,[10] 5,5′-dithiobis(2-nitrobenzoic acid) which reacts with thiolate ion at pH 7.2 to release the dianion of 2-nitro-5-mercaptobenzoic acid which has a maximum absorbance at 412 nm (extinction coefficient 14 150 dm^3 mol^{-1} cm^{-1}). For each thiol we standardised the procedure and obtained values of the extinction coefficient within 5% of the literature value.

Acknowledgements

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